γδT Cells in Lung Cancer Malignant Pleural Effusion: Friend? Foe?

In an era of rapid advances in immunotherapy for solid tumors, relatively little is known about the immune tumor microenvironment (iTME) of malignant pleural effusions (MPEs) and how to leverage the iTME for an overall antitumor effect.

In this issue of the *Journal*, Wei and colleagues (pp. 174–184) report the results of a deeper investigation into the iTME of lung cancer MPE using a murine model (1). In particular, they focus on the role of a particular subpopulation of lymphocytes, termed γδT cells, a population that has not been well studied in the context of MPE.

γδT cells have a unique T-cell receptor structure that is composed of two glycoprotein chains (one γ chain and one δ chain), in contrast to the more abundant and well-studied population of αβT cells. There are two major subsets, distinguished by their Vδ chain. Vδ1 T cells are found in the thymus and periphery and react to a variety of stress-related antigens (e.g., heat shock proteins), whereas Vδ2 T cells are the predominant subset in the blood (2). γδT cells have some key differences from αβT cells that may translate to enhanced activity against infections and cancers: *1*) they do not require antigen processing, *2*) they can recognize antigen independently of major histocompatibility complex (MHC) peptide presentation, *3*) they can recognize a broad range of antigens (most prominently lipid antigens), *4*) they display attributes of both innate and adaptive immunity, *5*) they can be activated rapidly (3), *6*) their cytotoxicity is exerted through a variety of mechanisms (4), and *7*) they can present antigen themselves, similarly to dendritic cells, and stimulate other immune cells (5).

γδT cells have demonstrated antitumor activity in preclinical models of various solid cancers. They have also shown promise in a few clinical trials (6). Both γδ1 and γδ2 T cells are able to lyse tumor cells *ex vivo* (7, 8) and express chemokine receptors that augment tumor homing (9). A particular subset of γδT cells, Vγ9Vδ2T cells, have drawn interest for tumor immunotherapy applications (10). However, some studies have found circumstances in which γδ T cells become exhausted (11), are suppressed through checkpoint molecules (12), or may even serve a protumor role. Some studies observed tumor progression when Vδ1 T cells exceeded Vδ2 T cells in number (normally, Vδ2 T cells are more numerous than Vδ1 T cells in the peripheral blood) (13, 14). In some reports, the presence of tumor-infiltrating γδT cells correlated with favorable outcomes in different cancers (15, 16), but in others the presence of γδ T cells correlated with worse clinical outcomes (17). Some studies have shown that γδ T cells can suppress other immune cells (18). IL-17A and IL-10 in the context of Vδ T cells are of particular interest. IL-17 is traditionally known as a proinflammatory cytokine, but it has been reported to play both anti- and protumor roles depending on the nature of the tumor microenvironment (19). Patients with lung cancer and lower levels of MPE IL-17 were found to have longer overall survival times (20). An increased frequency of IL-17$^+$ T cells was associated with prolonged survival of patients with lung cancer and MPE (21). IL-10, on the other hand, is traditionally known as an antiinflammatory cytokine and has been shown to promote MPE formation in mouse models (22). IL-10 has been reported to have an inverse relationship with IL-17 in MPEs (23). Only a few studies have examined the role of γδ T cells specifically in MPEs. Investigators in China detected γδ T cells in lung cancer MPEs, but these were on average lower in frequency than in matched peripheral blood samples (24). Other investigators studying breast cancer MPEs reported highly variable frequencies of γδ T cells in their samples (25).

Wei and colleagues attempted to elucidate the role of γδ T cells in MPEs, particularly in the context of IL-17 secretion. Having previously shown IL-10's suppression of antitumor responses in murine MPEs, they also investigated whether IL-17–producing γδ T cells (γδT17 cells) were suppressed by IL-10 in MPEs.

This study is really three ministudies in one. In their first study, the authors used a murine model of MPE. LLC or MC38 cancer cells were introduced intrapleurally into C57BL/6 wild-type (WT) mice or IL-10 knockout mice. About 2 weeks after injection, MPE, blood, and spleen samples were collected and analyzed via flow cytometry. In the second study, the authors took γδ T cells purified from mouse spleens, differentiated them for 3 days *in vitro*, and analyzed proliferation and cytokine production phenotype by flow cytometry. In the third study, they focused on the effect of antibody-based depletion of γδ T cells in the murine LLC MPE model.

The authors' main conclusion is that IL-10 deficiency increases γδ T cells in MPEs via enhanced proliferation, and promotes γδ T-cell production of IL-17A via upregulation of a transcription factor, RORγt. Following are some of the key observations of their study:

1) The authors found an increased frequency of δT17 cells and greater amounts of IL-17A in MPEs, which were enhanced in IL-10 deficiency. The majority of MPE γδ T cells had an activated, effector memory phenotype. Interestingly, IL-10 deficiency led to lower expression of FasL and NKG2D on γδ T cells.

2) The mechanism of IL-10 deficiency leading to enhanced MPE γδ T cells was due to enhanced proliferation, not enhanced trafficking. IL-10 deficiency further augmented proliferation.

3) The transcription factor RORγt (which is involved in the differentiation of T-helper cell type 17 [Th17] cells) was expressed at higher levels in IL-10$^{-/-}$ γδ T cells than in their WT counterparts. Other transcription factors (Tbet and IRF4) showed decreased expression in IL-10$^{-/-}$ γδ T cells.

4) Overall, IL-10$^{-/-}$ mice had smaller MPE volumes and survived longer with MPEs than WT mice. Antibody depletion of γδ T cells decreased the survival time of IL-10$^{-/-}$ MPE mice, but

not that of WT MPE mice. γδ T-cell depletion increased the volumes of MPE fluid in WT mice. The volumes were even greater in IL-$10^{-/-}$ mice.

These observations confirm other reports that the cytokine milieu could impact the ability of MPE T cells—specifically, the population of γδ T cells, with their inherent advantages over conventional T cells—to have an antitumor effect. Delivery of IL-17A into MPEs combined with blockade of IL-10 should be tested in light of these findings. Theoretically, this could also be accomplished by engineering activated T cells and adoptively transferring them into the pleural space.

The authors rightly acknowledge the controversies that remain in the field, including whether γδ T cells are pro- or antitumor or both, depending on what are still unknown circumstances. They propose that this is due to an interaction between γδT17 cells and other immune cells, but do not directly clarify this through their studies. Furthermore, they acknowledge that both γδT17 and Th1 cells were increased in IL-10–deficient MPEs, but these cells traditionally serve opposing roles in MPE formation.

Two limitations of this study are worth mentioning: *1*) FasL and NKG2D are markers that are known to be expressed on activated T cells, so it seems a bit counterintuitive that IL-$10^{-/-}$ γδ T cells were found to be more active but showed decreased expression of these markers; and *2*) the study was focused on the murine immune system, which is known to differ from the human system with regard to both innate and adaptive immunity (26). Overall, this study provides insight into a relatively unclear topic, but the results need to be validated before they can be generalized to human MPEs. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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